SMALLPOX AND RELATED POXVIRUS INFECTIONS IN THE SIMIAN HOST

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I. Introduction

A sense of complacency resulting from the apparent control of smallpox may be one of the factors responsible for the lack of interest in basic studies with its etiological agent. The constant vigilance practiced by public health authorities to prevent the importation of smallpox and the prompt vaccination of suspected contacts have been significant deterrents to its spread in the Western Hemisphere. Nevertheless, smallpox remains a continual threat to man, as evidenced by its endemicity in certain localities of the Middle and Far East, by occasional outbreaks of epidemic proportion in those areas, and by sporadic cases of the disease throughout the world. The disease persists, although vaccination against smallpox has been practiced for over 160 years.

Although smallpox infection of man has been described in detail from the standpoint of symptomatology, diagnosis, pathology, immunity, and mortality rates of epidemics, nonetheless there are areas in which our understanding of this disease requires elucidation. For example, the site or sites of initial multiplication of the virus, the pathways of virus dissemination during incubation and disease, the duration of protection following vaccination, the pathogenesis of the vaccination process, and the evaluation of chemotherapeutic agents are some aspects of the disease that may be resolved through the use of an experimental host.

The study of experimental smallpox, however, has been limited because of the narrow range of animals that are susceptible to the virus. Monkeys appear to be the only experimental animal in which the pattern of disease is similar to that of human infections. Although some fundamental studies were performed with the virus of smallpox in this host more than 50 years ago (11), relatively little work has been done in subsequent years. Most of the investigations have been concerned with cross-immunity relationships and with attempts to convert smallpox virus to vaccinia virus.

It is the purpose of this article, therefore, to review the pertinent literature on smallpox and related poxvirus infections of the simian host, to define some of the obstacles inherent to the study of poxviruses in monkeys, and to stimulate further basic investigation of these viruses.

II. Variola-Vaccinia Group of Poxviruses

"Poxvirus" has been adopted as a convenient term to denote a number of viruses which have many features in common, although they comprise several immunologically unrelated subgroups (3). The criteria for inclusion in the group are: (i) large virus particles of rounded quadrangular form, with the longest diameter in the range of 200 to 250 mμ; and (ii) predilection for infection of skin epithelium, with formation of cytoplasmic inclusion bodies (24). The close immunological relationship of certain viruses serves as the basis for the formation of a subgroup of the poxviruses, the variola-vaccinia group. Members of this group are variola (smallpox), alastrim, rabbitpox, vaccinia, monkeypox, cowpox, and ectromelia (mousepox). Because of the scarcity of published information regarding the infectivity of rabbitpox, cowpox, and mousepox viruses for the simian host, only casual reference will be made to these viruses.

In addition to the property of related anti-

TABLE 1. *Susceptibility of monkeys to related poxviruses*

Virus and host species	Route of inoculation[a]	Signs of infection[b]	Reference
Variola:			
Macaca cynomolgus	S, Sc, C, MM, IC, R	LL, F, GE	11, 20
Macaca irus	R, IT, IN	F, GE	38, —[d]
Saimiri sciureus	R	F, GE	—
Macaca sinicus	ITT	OR, F, GE	59
Cercopithecus callithrix	ITT, S	OR, F, GE	60, 69
Macaca rhesus	SC, IN, ID-IP, R, O	LL, F, GE	—, 53, 69
Macaca nemestrinus	S, IT, O, IV	LL, F, GE	11, 40, 58
Cercopithicus pathas	S	LL	60
"Macacus"	SC, S	LL, F, GE	4, 30, 46, 61
"Rhoesus"	SC, S	LL, F, GE	17, 36, 46, 61
"Cercopithecus"	O, ID	LL, GE	70
"Large African monkey"	SC	LL	30
"Java"	SC	LL	49
Simia satyrus	S, R?	LL, GE	10, 11
Alastrim:			
Macaca rhesus	IV, SC	LL, F	16, 19, 44
Macaca cynomolgus	S	LL	44
Macaca sinicus	SC	None[c]	63
Cercopithecus sebacus	S, IV	LL, F, GE	40, 65
"Monkey"	ICA	GE	21
"Rhoesus"	S	LL	36
Vaccinia:			
Macaca cynomolgus	IC, SC, S, C, MM	LL, E	1, 11, 20
Cercopithecus sebacus	S	LL	40
Cercopithecus mona	SC	LL	56
Cercopithecus albigularis	ID	LL, E	15
Cebus apella	SC	LL	56
"Rhesus"	SC	LL	37, 56
"Java"	O	LL	7
"Cynocephale"	S	LL, F	69
"Bonnet"	SC	LL, F	7
Macaca irus	R	F, D	—

[a] SC = subcutaneous, S = scarification, C = cornea, MM = mucous membranes, R = respiratory, IC = intracerebral, IT = intratracheal, ITT = intratesticular, O = oral, ID = intradermal, ICA = intracardial, IP = intraperitoneal.

[b] LL = local lesion, F = fever, GE = generalized exanthem, OR = orchitis, E = encephalitis, and D = death.

[c] There is a question as to whether the virus was alastrim.

[d] — = N. Hahon, *unpublished data*

genicity shared by members of the variola-vaccinia group, the ability to form lesions or pocks upon inoculation of the chorioallantoic membrane of embryonated eggs by each of the viruses serves as another point of similarity. Individually, the viruses appear to differ from each other in their range of infectivity. Variola and alastrim have a limited range of pathogenicity for experimental animals but are transmissible naturally to man. Cowpox and rabbitpox are natural diseases of cows and rabbits. The former is transmissible to man; the latter has not been reported to be transmissible to man. Mousepox and monkeypox are natural diseases of the species for which they are named. The former is not transmissible to humans and the pathogenicity of the latter for man has not been determined. Although vaccinia virus possesses the widest range of infectivity for animals of all the viruses in the group, it is unique in that it does not occur naturally.

III. Nature of Poxvirus Infections in Simian Host

A. Clinical Disease in Relation to Route of Virus Inoculation

Monkeys may be infected by almost any route of inoculation with the poxviruses, variola, alastrim, and vaccinia, but the reaction may vary from a local lesion at the site of inoculation to a generalized exanthem (Table 1). The route of virus injection, the strain of virus, and the species of monkey, however, may influence the response of the host to infection, either individually or concertedly.

1. Variola. In general, simian smallpox is a mild infection of approximately 2 weeks' duration whether induced by inoculum consisting of human scab material or laboratory-passaged virus (Table 2). The entry of virus into the host is followed by an incubation period of 5 to 7 days and a period of disease that lasts about 8 days. In this respect, the nature of smallpox infection of the monkey appears to conform to inoculated smallpox seen in man (11). The dermal inoculation of the virus in humans usually produces a local lesion with fever commencing on the seventh or the eighth day and a generalized exanthem on the ninth or tenth day. In man, the incubation period is shorter and the disease is generally milder than in the naturally acquired infection, but exceptions have been noted (68).

TABLE 2. *Comparative occurrence of exanthem in Macaca irus after intratracheal inoculation of the Yamada and Kali-Muthu strains of variola virus*

Virus quantity inoculated, infectious units[a]	Proportion of inoculated animals developing exanthem	
	Yamada (egg-passaged)	Kali-Muthu (human scab preparation)
3.0×10^4	2/3[b]	1/3
3.0×10^3	2/4	1/4
3.0×10^2	3/3	0/3
3.0×10^1	1/3	0/2
Total no. infected	8/13	2/12
Per cent infected	61	16

[a] Enumeration of pocks formed on the chorioallantoic membrane of embryonated eggs.

[b] Numerator = number of animals with exanthem, denominator = number of animals inoculated.

a. Intradermal. The clinical disease in monkeys, following dermal inoculation with variola virus, may also exemplify the infection after other routes of injection. The overt signs of smallpox infection in the monkey may consist of (i) a local lesion at the site of dermal inoculation, (ii) vague constitutional disturbances, (iii) a slight or marked elevation of temperature, and (iv) an exanthem of varied intensity.

A reaction appears on the skin at the site of inoculation in 3 days, and, on the sixth to seventh day, when the primary lesion is most active, body temperature rises and may peak at 105 F or higher (11). Other constitutional signs that may be manifested are anorexia, listlessness, coughing, or a loss of appetite. The febrile reaction precedes the appearance of exanthem by 24 to 48 hr. The skin eruption is noted between the eighth and tenth days after inoculation, but the extent of the exanthem varies markedly; in some animals one lesion may be present; in others more than 100 pocks are seen (Fig. 1 and 2). The distribution of the exanthem shows a partiality for certain regions; the face is most often the site of initial eruption. In the order of appearance elsewhere, the eruption is present on the wrists, scrotum of the male, region about the anus, base of the tail, palms, soles, and inner region of the arms and thighs. The trunk and the outer surfaces of the limbs are rarely affected. The evolution of the exanthem is relatively constant.

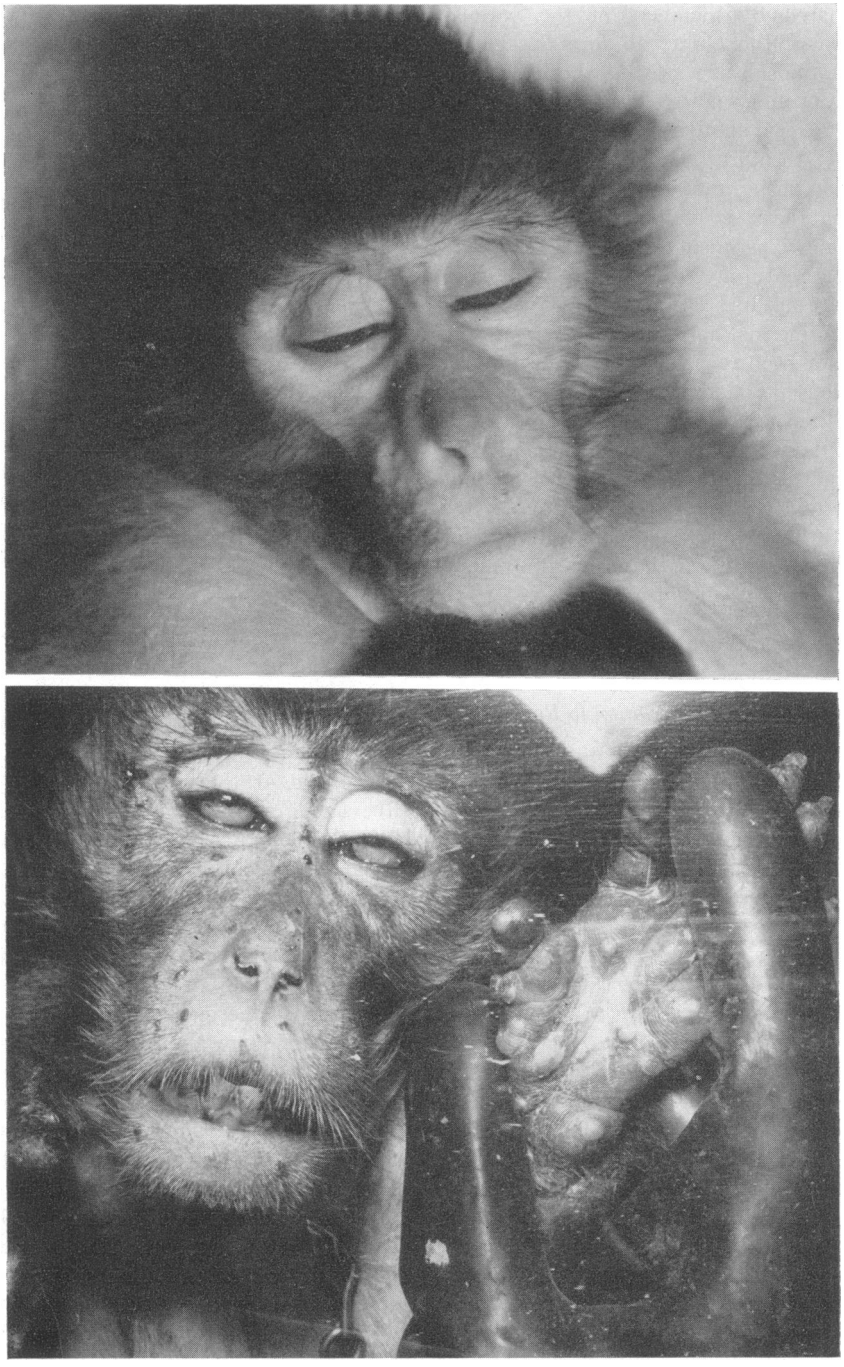

FIG. 1. *Top—A mild exanthem in Macaca rhesus following exposure to air-borne variola virus.*

FIG. 2. *Bottom—A severe exanthem in Macaca irus following intratracheal inoculation with variola virus.*

The eruption first appears as minute pink papules, rarely exceeding 1 mm in diameter. On the next day, this papule is larger and shows a vesicular structure. The fluid contents of the lesion become cloudy on the following day, and the lesion dries in another 24 hr. The exanthem, therefore, has its complete evolution in about 4 days. In some animals, however, the lesions pass through a longer period of development. The general exanthem usually lasts 5 days; the first 2 are employed in growth and the last 2 in healing.

Cursory observation of the cellular elements of the blood of monkeys infected with variola indicates a disturbance in leukocyte equilibrium characterized by a polymorphonuclear, followed by a mononuclear, leukocytosis (46). Although Bleyer (8) reported the finding of corpses of monkeys with variolous lesions in a natural epidemic of smallpox, a perusal of the literature shows that the mortality of monkeys infected with smallpox is negligible. Reports of experimental infections of monkeys with variola virus and subsequent death of the animals were attributed to intercurrent disease, i.e., streptococcal septicemia and tuberculosis (11).

Although infections of alastrim in monkeys have not been described in the detail accorded smallpox, the pattern of disease appears to be similar.

b. Respiratory. Exposure of monkeys to air-borne virus results in a disease pattern similar to that following dermal inoculation except that there is no local lesion (38). In these animals, a viremic period was detected both in the incubation and disease periods, and an immunological response, in the nature of neutralizing and hemagglutination-inhibition antibodies in the serum, was noted shortly after the appearance of the exanthem.

c. Other routes. A slight alteration from the pattern of infection after dermal injection may result after inoculation of the mucous membranes with variola virus. A general exanthem may occur in approximately 10% of monkeys; in contrast, an eruption develops in 70 to 80% of animals after inoculation of the skin (11). There is no abrupt elevation of temperature; listlessness and anorexia are absent.

After corneal inoculation, the lesion differs from the lesion of the skin in that vesicle formation does not occur. On the eighth day a general eruption may follow corneal injection; similarly, an eruption may follow intravenous inoculation, but it appears in 5 days (66). Orchitis was an additional feature of disease noted after intratesticular injection (59, 60). The introduction of high concentrations of virus directly into the alimentary tract of monkeys was characterized by a slight elevation of temperature and a rise in serum-neutralizing antibody (N. Hahon, *unpublished data*). An exanthem was not produced.

The clinical disease in monkeys, inoculated with virus by other routes (Table 1), is not unlike that recorded after a dermal injection.

2. Vaccinia. a. Parenteral. In monkeys, it is easier to distinguish the pattern of infection with vaccinia from that of variola than it is to distinguish variola from alastrim. The introduction of vaccinia virus in monkeys results in a lesion at the site of inoculation, but the febrile reaction is not as definite as in variola. A slight fever, which rarely exceeds 104 F, may occur between the sixth and ninth days after inoculation. The general condition of the animal is not disturbed, and generalized exanthem is absent. The inoculation of the cornea with vaccinia virus gives rise to a typical keratitis. The lesion is comparable to that following inoculation of the rabbit's cornea (64). Lesions appear at the site of vaccinial injection of mucous membranes that are similar to the skin lesions. Again there is no generalized eruption (11).

It is noteworthy that the inoculation of monkeys with vaccinia virus does not incite a general exanthem. Brinckerhoff and Tyzzer (11) and Clearkin (15) reported that they never observed a general eruption in the simian host after inoculation with vaccinia virus. The former investigators contended that variola and vaccinia viruses may be differentiated by their infectivity response in monkeys as follows: variola virus produces a local lesion accompanied by an exanthem and is infectious by the air-borne route; vaccinia virus produces a local lesion without exanthem and is not infective in the air-borne state.

b. Respiratory. Recent studies revealed the susceptiblity of *Macaca irus* to concentrated aerosols of three different strains of vaccinia virus: International Health Division (IHD), Western Reserve, and Downie (N. Hahon and M. McGavran, *unpublished data*). Elevated temperatures were noted in animals within 4 days following virus exposure, with temperature peaks of 105 F or greater occurring 2 days later. Definite

signs of constitutional disturbance were observed. Deaths occurred within 1 week, and were more frequent in animals exposed to Western Reserve or Downie strains than in animals exposed to the IHD strain. Neutralizing antibody was found in sera of surviving animals. There was no sign of an exanthem in any of the exposed animals. It appears, therefore, that monkeys are susceptible to air-borne vaccinia virus, but differ from monkeys infected with variola virus with respect to the manifestation of an exanthem.

3. Monkeypox. a. Natural. The disease first observed in a colony of captive monkeys was marked by umbilicated cutaneous lesions which were similar to those reported for human beings with cases of variola (54). Of those animals showing observable physical signs of infection, a high percentage were *Macaca philippinensis*. Only a few *Macaca mulatta* exhibited clinical pox lesions. No signs of illness were noticed prior to the appearance of cutaneous lesions, but a few animals showed edema of the face.

Cutaneous manifestation occurred as multiple, discrete papules; many showed dark reddish-brown umbilicated centers. The papules evoluted to pustules and later, to reddish-brown crusts which fell off in 7 to 10 days leaving a small scar. Eruptions appeared on any portion of the cutaneous surface; lateral and dorsal aspects of the tongue; oral, pharyngeal, laryngeal, and tracheal mucosa; but were most commonly observed on the buttocks, hands, feet, and hind limbs. The number of deaths due to the disease was less than 0.5%. The eruptions in infants and in adults with fatal cases had a tendency to become hemorrhagic.

Sera from infected animals contained hemagglutination-inhibition and complement-fixation antibodies (52).

b. Respiratory. The disease pattern observed in *M. irus* exposed to air-borne monkeypox virus was similar in many respects to that recounted with vaccinia virus. The clinical features of disease were: (i) onset of fever on the fourth to fifth days with subsequent elevation of temperature to 105 F, (ii) constitutional signs, anorexia, coughing, and loss of appetite, and (iii) the occurrence of an exanthem on the ninth day after exposure and the occasional appearance of edema of the face. Neutralizing antibody was detected in sera of animals (N. Hahon and M. McGavran, *unpublished data*).

c. Other routes. Local lesions were observed in monkeys after scarification of the skin; a systemic pox was manifested in monkeys after intravenous injection of virus (52).

4. Factors affecting resistance. A few investigators have studied the effect of altering the simian host's physiological balance on its response to poxviruses. Eckstein and Sarvan (29) observed the effect of starvation on the development of vaccinia in monkeys after intracutaneous injection and noted that the reaction was delayed, was more necrotic, and healed more slowly. The removal of the spleen from animals also retarded the vaccinial reaction on the skin.

A comparative study of the pattern of disease in *M. irus* which had been either splenectomized or treated with cortisone prior to intranasal inoculation with variola virus showed that their reaction was similar to that of the control group of animals (N. Hahon, *unpublished data*). The manifestation of disease, in the form of a generalized exanthem, was of comparable intensity and appeared at approximately the same time in all three groups of animals.

B. Species Susceptibility

The range of animals that are susceptible to infection with variola and alastrim viruses is limited; monkeys are the preferred experimental hosts. Although vaccinia virus shows a wider range of animal susceptibility than almost any other known infection, the rabbit has been employed most often as the experimental animal. A survey of the literature on infectivity studies with the poxviruses in monkeys indicates (Table 1) that various monkey species are susceptible to variola, alastrim, and vaccinia viruses following diverse routes of virus inoculation. Unfortunately, the exact number of species in this list is uncertain, because the monkeys used by different investigators were not identified completely. Furthermore, there is no apparent distinction, based on a quantitative study, to distinguish the species of monkeys most susceptible to members of the variola-vaccinia group of poxviruses.

Brinckerhoff and Tyzzer (11) suggested that different species of monkeys may show different degrees of susceptiblity to the contagium of smallpox. Because New World monkeys differ in many respects from their relatives of the Old World, these authors believed that the former may be more susceptible to smallpox than the

latter. There is some experimental evidence, based on a comparative study of two species of monkeys, to justify the view that different sepcies may vary in their susceptibility to smallpox virus. When *Macaca rhesus* and *M. irus* were exposed to uniform quantities of air-borne variola virus, 50% of *M. rhesus* were infected, as evidenced by an elevation of body temperature and specific antibody response, but without the occurrence of an exanthem; in contrast, 80% of *M. irus* were infected and all infected animals exhibited an exanthem (N. Hahon, *unpublished data*). This finding does not imply that generalized variola may not occur in *M. rhesus*, because it has been noted that an exanthem may be elicited if the virus dose is sufficiently large (Fig. 1).

Because rhesus monkeys are generally collected in India and are mostly inhabitants of crowded urban areas, they may come in contact with smallpox virus through human cases of smallpox, fomites, or air-borne virus. The presence of antibody, incited by proximity with the virus, may explain the mild or irregular response of *M. rhesus* to subsequent exposure to the virus in experimental studies. The experience of Peebles et al. (51), with monkeys possessing serological evidence of infection to measles virus, supports this supposition.

That the susceptiblity of different species of monkeys and their lack of uniformity of response to infection with related poxviruses may be dependent on a previous exposure to an antigenically related agent in their natural environment receives further support from the recent discoveries of the existence of a natural pox disease of monkeys (52, 54). One of the etiological agents has been shown to be antigenically related to the variola-vaccinia group of poxviruses (52).

The problem regarding the susceptibility of the simian host to the poxviruses might be clarified, if a survey were carried out to determine the extent of specific antibodies to the poxviruses that are present in different monkey populations.

C. Pathogenicity and Virulence of Poxvirus Strains

Variations of mortality rates with diverse clinical forms of disease have been reported in human epidemics of variola (23, 42, 44, 57). In the Minneapolis outbreak of 1924 and 1925, Sweitzer and Ikeda (57) observed that the mortality rates with respect to the clinical forms of disease in a single hospital group were as follows: 225 discrete type, 14 deaths; 151 confluent type, 68 deaths; 144 hemorrhagic type, 113 deaths; and 51 purpuric type, 51 deaths. Differences in the virulence of different strains of variola virus from cases of varying severity in the same epidemic could not be distinguished following inoculation of monkeys (46). Because of the paucity of data available, one can only speculate on the cause of the different manifestations of disease within the same epidemic. It may be related to slight changes in the virulence of the virus, the general health and nutrition of the population, or some undetermined factor.

In nature, strains of smallpox virus of greater or lesser lethality occur which appear to remain unaltered on passage and give rise to epidemics of such characteristic mortalities as to permit differentiation into classical smallpox (variola major) with a mortality rate of 20 to 30% and alastrim (variola minor) with a mortality rate of less than 1%. Epidemiological studies have shown that the two clinical forms are independent entities. Although the consistently low death rate cannot be foreseen at the onset of a smallpox epidemic (9), certain clinical differences may exist between variola and alastrim in man; these differences have been summarized by De Jong (21).

In general, laboratory techniques for distinguishing between the viruses of variola and alastrim have failed to reveal constant differences with regard to the histology of skin lesions (21, 28), the lesions produced on the chorioallantoic membrane of embryonated eggs (28), cross-immunity experiments in monkeys (40), serum-neutralization, complement-fixation, and hemagglutination-inhibition tests (25, 27, 47), by the double-diffusion precipitation technique (35), and the use of laboratory animals (22). Within recent years, however, the findings from studies of the behavior of smallpox and alastrim viruses in embryonated eggs offer a means of differentiating them. Smallpox persists longer than alastrim on the chorioallantoic membrane (22), as measured by mortality rates; smallpox was more pathogenic for the chick embryo than was alastrim (39); and lesions were produced by smallpox in eggs held at 38 to 38.5 C for 2 to 3 days, but no lesions were formed by alastrim at this temperature (48).

In their comprehensive studies with smallpox, Magrath and Brinckerhoff (46) noted that dif-

TABLE 3. *Comparative pathogenicity of strains of variola virus in Macaca rhesus after intranasal inoculation*

Virus strain[a]	Dose, infectious units[b] ($\times 10^7$)	Cutaneous lesions		
		Face	Limbs	Trunk
Harper	1.0	+	+	+
Harper	1.0	+	+	+
Stillwell	5.0	+	−	−
Stillwell	7.5	+	+	−
Yamada	3.0	Died[c]		
Yamada	3.0	+	−	−
Lee	2.8	+	−	−
Lee	2.8	Died[c]		
Kim	5.3	+	+	−
Kim	5.3	+	−	−
Gassman	0.5	+	+	+
Gassman	0.5	+	+	+

[a] Harper, Stillwell, and Gassman strains were isolated in the Far East in 1951 from fatal cases of smallpox. The Yamada strain was isolated in 1946 from a Japanese patient with a moderately severe case of smallpox. Lee and Kim strains were isolated from a Korean outbreak of smallpox in 1946; 14 deaths occurred in 40 cases.

[b] Enumeration of pocks formed on the chorioallantoic membrane of embryonated eggs.

[c] Death of animal was not related to the virus.

ferent strains of variola virus from different epidemics exhibited varied degrees of virulence for the monkey. Virus from epidemics of clinically severe variola produced lesions that were more likely to be followed by a general exanthem. Somewhat similar observations were recorded by Gordon (36); viruses from a case of alastrim and from a case of confluent variola were found to be infective for the rhesus monkey when inoculated by scarification. There was, however, a pronounced difference in virulence; the lesion produced by a virus of confluent smallpox was more severe than that produced by the virus of alastrim.

Several experimental studies suggest that differences in the virulence for monkeys do exist between strains of both variola and alastrim viruses, and may be dependent on geographical origin of the strains. In one study, this was evident when two strains of variola virus, isolated from different geographic areas, were compared for pathogenicity and virulence by intratracheal inoculation of *M. irus* with equivalent doses of virus (N. Hahon, *unpublished data*). One of the strains, Yamada, originated in the Far East; the other, Kali-Muthu, was obtained from India. The results (Table 2) indicate that both strains were pathogenic for monkeys, but the Yamada strain was more virulent, as evidenced by the higher incidence of exanthem, than the Kali-Muthu strain. A comparison of the virulence of five different strains of variola virus from the Far East revealed that all strains induced an exanthem in monkeys following intranasal inoculation (Table 3). A distinction between strains on the basis of virulence (incidence of exanthem) was not possible (N. Hahon, *unpublished data*).

Similar findings with alastrim virus were reported by Horgan and Haseeb (40). From a review of the literature, they noted that all American and English strains of alastrim appeared to react fairly readily on the monkey but not on any other animal. An Indian strain, however, failed to produce a reaction; an African strain induced a primary response in monkeys only after intravenous injection of virus prior to scarification of skin. In their own studies, these investigators found that *Cercopithecus sebacus* (Sudan monkeys) were completely refractory to the Khartoum (African) strain of virus but fully susceptible to the St. Louis (American) strain of virus. Moreover, three strains of "Congo" alastrim from typical, mild cases showed a very low degree of infectivity for the same species of monkeys, and some animals were completely resistant (41). The possibility of variation in the susceptibility of individual monkeys was not excluded, but the evidence was regarded as strongly suggestive that differences in the strains of alastrim play a more important role.

This brief account of the literature provides suggestive evidence that variola and alastrim viruses may be differentiated by their pathogenic effects in the simian host and, further, that different degrees of pathogenicity and virulence for monkeys which appear to exist between strains of each of the viruses, may be related to geographical location. The incompleteness of experimental evidence, however, does not permit unreserved correlation of the numerous clinical varieties of human smallpox with the reaction noted in the monkey after virus inoculation.

This problem may be elucidated, in future studies, if the following factors are considered: dose, species of monkeys, screening of animal

sera for antibody to related poxviruses, route of inoculation, equivalent passage of virus strains in the same host, and, lastly, a complete history of the origin of the virus strains.

D. Pathology in Relation to Route of Virus Inoculation

The pathology of the skin lesions associated with variola, vaccinia, and alastrim infections in the simian host is considered to be similar to that recorded for human infections. The principal source of our knowledge regarding these infections in the monkey is attributable to the investigations of Brinckerhoff and Tyzzer (11), who in the early 1900's studied the tissue reactions of both variola and vaccinia viruses after different routes of inoculation. Since that time, little has been added by others to their classic description of the histopathology of these infections in the simian host.

1. Variola. a. Dermal. The macroscopic development of the primary lesion at the site of dermal inoculation with variola virus resulted after 3 days in an elevated lesion marked by a crust in the center of the lesion on the fifth day (11). The crust was surrounded by a "vesicular ring" and a zone of elevation and hyperemia at the periphery. After the eighth day, the lateral excursions of the lesion ceased and involution began; this process involved the further spread of the crust and the fading of hyperemia. Thereafter, normal epithelium grew slowly beneath the crust. The lymph nodes draining the area of the skin lesions increased in size on the fifth day and diminished on the tenth day. Macroscopic lesions of the viscera were absent.

Histologically, the evolution of the primary lesion was characterized by a combination of degenerative, exudative, and reparative processes. From the third day onward, there was extensive degeneration of epithelial cells, which was preceded by swelling and proliferation. The collection of fluid between epithelial cells led to the formation of definite cavities. The polymorphonuclear leukocytes passed into the fluid of the vesicle, and their increasing numbers gave the lesion the macroscopic character of a pustule. The vesicle was formed in the epidermis, but the corium showed enlargement and proliferation of endothelial cells of lymphatics and blood vessels. Later, a definite edema of the corium and of the adjacent subcutaneous tissue occurred, with evidence of necrosis beneath the lesion. In the latter stages, the lesion underwent repair; the epithelium grew in from the sides and upward from the hair sheaths to close the defect caused by the variolous process.

The process seen in the epithelium in the development of the exanthem was similar to that of the primary lesion. In the corium, however, edema and necrosis were absent.

b. Corneal. Inoculation of the cornea of the monkey with variola virus produced a specific lesion characterized by swelling, proliferation, and varying degrees of degeneration of the epithelial cells (11). The lesion resulted in less destruction of the corneal epithelium than followed inoculation of the cornea of the monkey with vaccinia virus. The lesion on the cornea differed from the variolous lesions on the skin of the monkey in that exudation did not play as prominent a part, and true vesicles did not form. Guarnieri bodies were present in the lesion up to 11 days after inoculation.

c. Mucous membranes. The primary lesion formed by inoculation of mucous membranes of the lip, nose, or palate of the monkey with variola virus was similar, cytologically and histologically, to that which followed variolation of the skin (11). The lesions produced on the mucous membranes did not form a crust. Guarnieri bodies in both cytoplasmic and nuclear phases were present in the lesions.

Inoculation of the mucous membranes of the monkey's trachea with variola virus was followed by the development of a variolous lesion which was similar to that produced on other mucous membranes (11). A variolous lesion may develop in the bronchi and be associated with a pneumonia. The presence of bronchopneumonia in monkeys exposed to air-borne variola virus could not be demonstrated, although the development of the exanthem was identical to the lesions described after dermal inoculation of monkeys (38). The role of variola virus in the pathological process noted in the lungs was inconclusive because of the presence of an underlying pneumonitis observed in the control animals.

2. Alastrim. The pathology of alastrim infections in the monkey has not been described in the detail accorded variola or vaccinia. On the basis of the appearance of dermal lesions, alastrim infections are similar to variola infections in the simian host (44). Histological differences be-

tween variola and alastrim, based on morphological and tinctorial characteristics of inclusions, have been emphasized by Torres (62), who studied the appearance of the Malpighian cells in portions of skin removed at biopsy from human patients suffering from variola and alastrim, as well as in the skin of rhesus monkeys infected with the two viruses. The differences, seen in inclusions of the two diseases, were not observed in human lesions by De Jong (21).

3. Vaccinia. a. Dermal. The inoculation of the skin of monkeys with vaccinia virus was followed by the development of a lesion at the site of injection. The histological changes in the skin of the monkey infected with vaccinia were very similar to those produced by variola virus (66). A vesicular change occurred in the epidermis, and the presence of Guarnieri bodies was noted. Lesions observed in the dermis were proliferation of the capillary endothelium and cellular infiltration. The histology of the axillary lymph nodes of monkeys vaccinated on the abdomen presented the same picture as that seen in variola-treated monkeys.

b. Corneal. Corneal inoculation of the monkey with vaccinia virus produced a lesion which was specific and comparable with that following the inoculation of the rabbit (66). The lesion was characterized chiefly by an early loss of epithelium at the site of inoculation, accompanied by the development of photophobia and conjunctivitis. Guarnieri bodies were present in the cells of the lesion.

c. Mucous membranes. Vaccination of the monkey upon the nasal, oral, or buccal mucosa gave rise to a true vaccinial lesion similar to that which followed vaccination of the skin (11). Guarnieri bodies were found only in the cytoplasm of the epithelial cells of the lesion. In variola lesions, both the cytoplasmic and nuclear bodies were noted.

d. Intracerebral. A number of investigators have studied the development of meningoencephalitis in monkeys after intracerebral inoculation with vaccinia virus; their findings are summarized by Van Rooyen and Rhodes (66). In the majority of studies, meningitis was the characteristic lesion produced by the intracranial injection of vaccinia virus, but lesions did not usually develop in the brain after intradermal inoculation.

e. Respiratory. Following exposure to airborne vaccinia virus, significant pathological alterations were observed in lung tissue of dead or moribund monkeys (N. Hahon and M. McGavran, *unpublished data*). The basic pulmonic response was an ulcerative bronchiolitis, bronchitis, and peribronchial inflammation to which was added a varying amount of virus pneumonia as evidenced by interstitial thickening and edema. Changes in lymphatic tissues consisted of cortical necrosis of the tracheobronchial and mediastinal lymph nodes and acute splenic hyperplasia. In a few instances, intracytoplasmic inclusions were found.

4. Monkeypox. In naturally infected monkeys, the microscopic appearance of the skin lesion was characterized by proliferation of the epidermis followed by necrosis (54). Coagulation necrosis affected the formation of typical intraepidermal vesicles. Large confluent vesicles and pustules occurred infrequently. Intracytoplasmic inclusion bodies were numerous in epidermal cells along the sides of the skin lesions; eosinophilic intranuclear inclusions were noted occasionally, but never concurrently in a cell with intracytoplasmic inclusion bodies.

The visceral lesions found consistently in all monkeys with fatal cases and in sacrificed animals were foci of necrosis in lymphatic tissue; i.e., spleen, lymph nodes, and solitary follicles of the digestive tract.

E. Cross-Immunity Relationships

Cross-immunity or cross-protection tests performed in monkeys were the means by which information regarding the immunological relationships among variola, vaccinia, and alastrim viruses were established at the turn of the century (17, 20, 56). Subsequently, but to a limited extent, the protection afforded humans against smallpox by vaccination has been studied experimentally in monkeys in an attempt to define the parameters of the immune reaction.

As early as 1902, Roger and Weil (53) regarded both vaccinia and variola viruses as inoculable upon the monkey, but held that neither confers perfect immunity. In subsequent experiments with monkeys, it was determined that reciprocal immunity of variola and vaccinia was not well defined; the immunity conferred by inoculation of variola virus was less durable against vaccinia virus than the converse (11, 36, 46, 69).

In a series of cross-protection tests between

variola and vaccinia viruses, the observations of Horgan and Haseeb (40) indicated that, although cross immunity was strong, it was not completely reciprocal in most cases. The protection induced by vaccinia virus against both viruses was complete; that of variola was complete against itself, but varied from strong to complete against vaccinia. The immunity relationship between alastrim (St. Louis) and vaccinia viruses was comparable to that of variola and vaccinia.

In other studies with variola and alastrim viruses (40), it was also found that immunity was not fully reciprocal, but that the degree of protection induced by alastrim was greater than that by variola. This finding was of considerable interest, because the variola strain used was the most virulent for monkeys isolated in the Sudan, and the alastrim was incapable of producing more than a local reaction. In contrast to these observations, Leake and Force (44) reported complete reciprocal immunity between alastrim (West Indies) and variola viruses. Although strains of alastrim from different sources in Africa and the United States have been shown to be closely related immunologically, the immunity between strains was not fully reciprocal (41).

Some of the diversity of the experimental results regarding the protection conferred by one virus against another may be attributed or influenced by the following factors: (i) site of initial inoculation of virus, (ii) site of second inoculation of virus, (iii) total area inoculated, (iv) dose, or (v) species of monkeys.

The sites of virus inoculation, both initial and secondary, as they influence the immune reaction, have been exemplified by the observations of Brinckerhoff and Tyzzer (11). When vaccinia virus inoculated on the skin resulted in the formation of a lesion, protection was manifested against subsequent inoculation of the cornea with vaccinia virus. A variola lesion of the skin, however, did not protect against subsequent inoculation of the cornea with variola virus, but a variola lesion of the cornea protected against subsequent inoculation of the skin with variola virus.

When the cutaneous route was used, the production of immunity by variola virus against vaccinia virus was directly related to the extent of the primary reaction and for complete protection a well marked reaction over a considerable area of the skin appeared to be essential (40). The larger the area, the greater the possible immunizing dose, and the greater was the chance of a strong reaction.

The dose of the virus inoculum may be important also, but dependent on the strain of virus employed (40). The larger the amount of variola virus inoculated, the more complete was the resultant immunity to vaccinia virus. Small inocula of vaccinia virus appeared to be sufficient, however, because the virus had a greater capacity for proliferation in tissues of most animals.

The effect of the susceptibility of different species of monkeys to the poxviruses, and the variability of response following infection, has been referred to in a previous section.

The immunological relationships established by cross-protection tests, briefly reviewed here, were based predominantly on challenging the host with a cutaneous injection of virus. A more meaningful expression of the data obtained through the use of monkeys, if it were to be related to human experience, might be forthcoming if vaccinated animals were challenged in subsequent cross-immunity tests in a natural manner, i.e., by exposure to known quantities of air-borne virus.

F. Assessment and Duration of Immunity

Apart from the studies of Brinckerhoff and Tyzzer (11), information regarding the development of the immune state in monkeys after vaccination is meager. Inasmuch as the data reported by these investigators did not include the status of the antibody levels of vaccinated animals at the time of challenge, they should be considered preliminary, at best. Their findings on the development of immunity after inoculation of the skin of the monkey with vaccinia and variola viruses indicated that the immunity which accompanied the development of the vaccinia lesion and variola lesion became manifest between the sixth and the eleventh day, and between the fifth and the eighth day, respectively.

Only recently has any information been reported regarding the antibody response in the simian host following infection with a poxvirus (38). In monkeys exposed to air-borne variola virus, neutralizing and hemagglutinating-inhibition antibodies appeared in the serum on the tenth and eleventh days after exposure, respectively. Similar to findings reported with human cases of disease, neutralizing antibody preceded hemagglutinating-inhibition antibody, and both

were noted shortly after the appearance of the exanthem (41).

There is little experimental evidence of the duration of immunity following recovery from infection with variola virus, or of vaccinial immunity after vaccination of monkeys. Unpublished studies (N. Hahon) indicate that *M. irus*, vaccinated 1 year previously by the multiple pressure technique with commercial smallpox vaccine, were resistant to large doses of inhaled variola virus. The only manifestation noted in challenged monkeys was in the nature of an immunogenic response; a two- to fourfold rise in serum neutralizing antibody.

At present, the sum of our knowledge concerning the status of immunity in man and monkeys may be aptly summarized by the views of Buddingh (13), presented 10 years ago:

"The viruses of the pox group are generally considered as capable of stimulating a long lasting if not a permanent immunity. This impression has been largely based on the widespread practical application of vaccination as a protection against smallpox. When considered critically it is quite apparent that much information is still required for an adequate understanding of the factors which are operative in this phenomenon. No positive statements can be made as to the approximate range of the duration of the protection afforded by a primary vaccinial reaction against variola, and much still remains to be learned as to the persistence of circulating antibodies in relation to the duration of effective resistance."

G. Pathogenesis

The pathogenesis of the acute viral exanthems is one of the most perplexing problems of these diseases. Experimentally, efforts to study this problem have been hampered by the lack of either a suitable laboratory host or a sensitive method for detecting the etiological agent. Consequently, a paucity of experimental data on the nature of the processes involved, particularly during the incubation period, limits our understanding of the exanthems. The high infectivity and long incubation period of variola, rubella, measles, and varicella, followed by a sudden onset of symptoms and generalized skin rash, may be explained only by postulating a site or sites of primary multiplication of the virus.

In general, most hypotheses agree that the respiratory tract is the natural route of infection with variola virus (5, 6, 14, 26, 33, 43), but differ with respect to the specific sites of viral multiplication and pathways of dissemination. The earliest experimental evidence of the possible site of primary multiplcation of virus in smallpox was recorded by Brinckerhoff and Tyzzer (11). They inoculated monkeys intratracheally or by blowing variola virus into the lungs, and noted the occurrence of a variolous lesion in the bronchi. The infectious agent was found in the lungs, and it was suggested that the virus was capable of multiplying in the "deep tissues" of the respiratory tract. It was considered possible that such a lesion might develop unnoticed and serve as a focus for multiplication of the virus during the incubation period of disease. Concurrence in this view by Councilman (18) was based on studies in which a systemic infection of variola was rarely produced, as shown either by immunity or by exanthem, after inoculation of virus on the mucous membrane of the nose, mouth, palate, or on the cornea.

From human cases of smallpox, Paschen (50) reported the presence of virus in the throat of contacts during the incubation period. It was suggested that after multiplication in the throat, the lungs were invaded and, later, a secondary liberation of virus occurred which was carried by the blood to the skin and other organs.

The casual observations of MacCallum et al. (45), who recovered a small amount of virus from a swab inserted into the lung of a patient dead from smallpox, is equivocal evidence that lung tissue was a focus of virus infection. These investigators believed that the virus recovered from the lungs may have been due to contamination from blood or skin during the process of manipulation. Nevertheless, they proposed that variola virus in the course of the infectious process is inspired into the lungs, where the primary focus or lesion develops. Such a lesion may break through into the capillaries, or even externally, and empty into the bronchi. Thus, a large amount of virulent virus is expelled in a relatively short interval in the febrile pre-exanthem period. In a large proportion of cases, however, the patient becomes infectious when, as a result of the viremia, lesions appear on the palate, tongue, and pharyngeal or buccal mucosa. These lesions break down, and virus is freed into the oral cavity.

In a recent investigation, the course of smallpox infection was followed in *M. irus* after ex-

posure to air-borne variola virus (38). Such a study was feasible because (i) a quantitative method was available for detection of small amounts of virus in tissue by inoculation of the chorioallantoic membrane of the embryonated egg; (ii) as an experimental host, *M. irus* was susceptible to infection by air-borne virus; and (iii) the overt manifestation of smallpox in these monkeys resembled the disease described in humans. These circumstances offered a means of studying one of the viral exanthems which is assumed to be transmitted in nature via the respiratory tract.

A portion of the findings depicted in Fig. 3 reveals the interrelationships between the propagation of variola virus in the respiratory passages, the antibody response, and the clinical disease in the simian host. The most striking observation was the daily increase in viral content of the lungs and other respiratory tract tissues during the incubation period. The pattern of virus multiplication in the lungs corresponded to a typical growth curve. Virus proliferation reached its peak within 3 to 5 days and began to decrease before the onset of the febrile period. In the latter stages of disease, virus in the upper respiratory passages increased notably as the virus content of the lungs diminished, probably as a result of secondary foci of infection. The antibody response occurred soon after the appearance of the exanthem.

The findings of this study suggested that the pathogenesis of air-borne variola involves several sites of simultaneous viral multiplication and perhaps various means of dissemination in the simian host. The infection of monkeys by aerosolized virus may follow this sequence: Inspired virus is deposited in the respiratory tract, lodging predominantly in the alveolar tissue of the lungs. The lower respiratory tract constitutes the principal site of primary infection, in which virus multiplication follows a typical growth curve. Coincidental with virus proliferation in the primary site and shortly after exposure of the host, virus may be distributed to other tissues or organs by swallowing of impinged material or by lymphatic drainage of virus initially implanted along the upper respiratory tract. At the height of propagation in the lungs or even earlier, virus may be released into the blood stream and lymphatics or spread to a lesser degree to other host tissues by the mechanics of exhalation, coughing, and swallowing. Secondary sites of multiplication established in additional lymphoid tissue continue to release virus into the hemal system, resulting in its further dissemination to the upper respiratory tract, urogenital system, central nervous system, and skin, in which the characteristic exanthem is formed.

Despite the seemingly ideal circumstances which permit an experimental study of the pathogenesis of smallpox, it would be unwise to suggest smallpox as a standard model for other exanthems. This view appears justified, as in the case of mousepox, which has been proposed as a model of the viral exanthems (31). Although there is a resemblance between the disease features of mousepox and smallpox, the portal of virus entry is different. As noted previously, the portal of entry of naturally acquired infections of smallpox and possibly other exanthems is through the respiratory tract; in mice, the virus of mousepox enters through a small abrasion of the skin, which becomes the site of primary multiplication. The comprehensive and revealing studies by Fenner (32), which depict a scheme for the pathogenesis of mousepox, may be pertinent to our understanding of the course of infection with poxviruses that are introduced directly into the skin of the host.

According to Briody (12), there is an indication that the natural portal of entry of mousepox virus may be through the respiratory passages. If this is true, then the scheme of infection with mousepox requires reevaluation. It is evident, therefore, that the premature acceptance of a standard model to explain the pathogenesis of the exanthems may confuse rather than clarify our knowledge of this problem.

IV. Appraisal of Simian Smallpox in Relation to Public Health

Because observations recorded in the literature indicate a natural relationship between smallpox and monkeys, it is worthwhile to examine these reports and to consider their implications. The natural occurrence of smallpox in monkeys was observed by Schmidt (55) and later by Bleyer (8), who noted the mild form of smallpox (alastrim) among the natives of the region of the Upper Uruguay River in Brazil. The disease spread to monkeys (*Mycetes* and *Cebus* species), which it killed in large numbers. Corpses were found under trees, and the bodies of sick or dead ani-

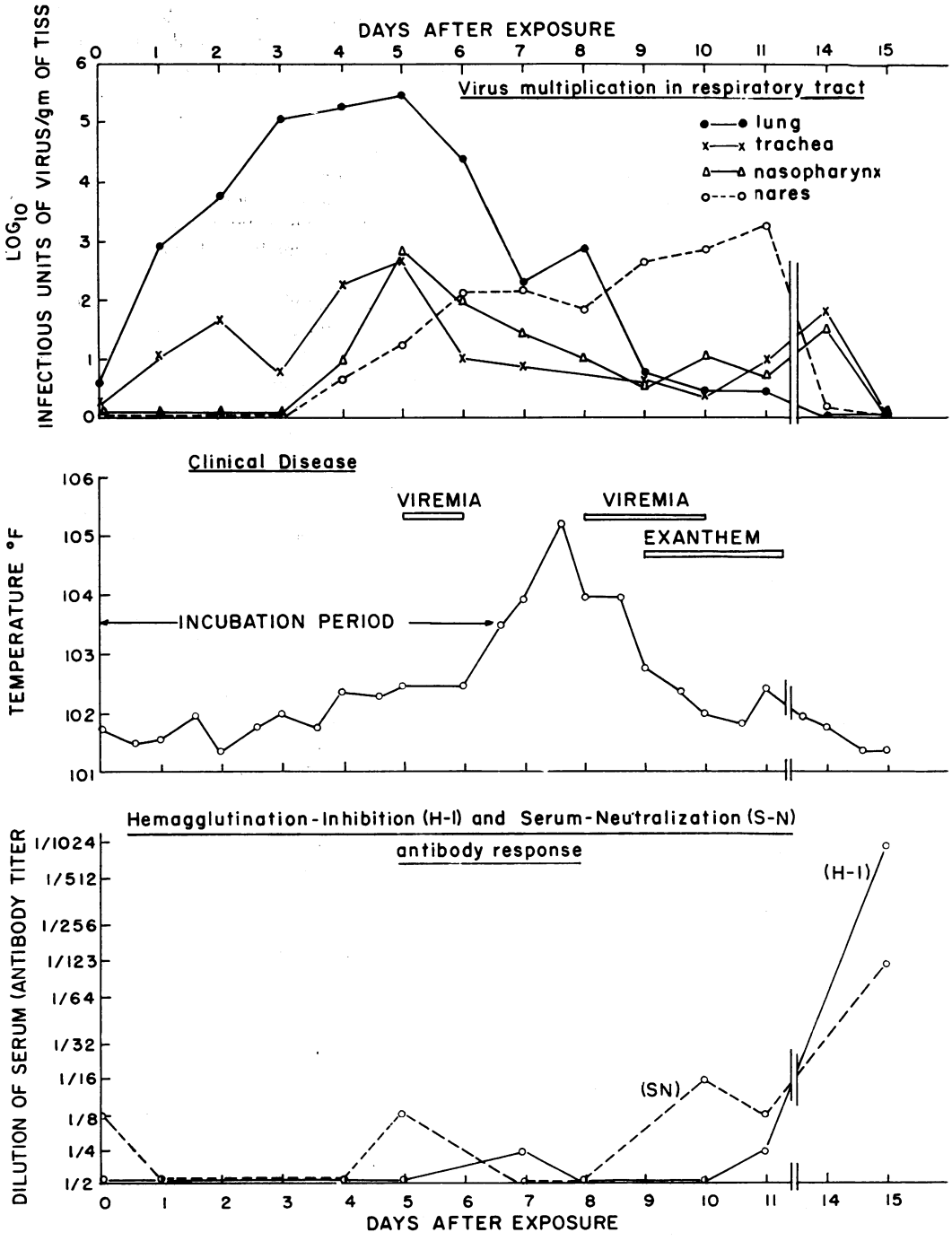

FIG. 3. *Pathogenesis of variola in Macaca irus exposed to air-borne virus*

mals were covered with variolous pustules. Councilman (18) refers to a similar occurrence of smallpox in man and monkeys near Panama in 1841. Among the reports of a natural exanthem occurring in primates other than monkeys is that of Fox and Weidman (34), who observed an acute papular and desquamative exanthem in an orangutan. During an epidemic of smallpox in

1949, a case of variola with a typical rash was reported in an orangutan (*Simia satyrus*) in a zoo (10). Animals of the same species caged nearby were vaccinated and the disease was confined. The experimental infection of monkeys with human isolates of air-borne variola virus serves as additional evidence that smallpox may be transmitted naturally from man to primates (38).

The transmission of variola virus from monkey to man remains undemonstrated, but some evidence for it is available based on an observation of a natural event. An excerpt from a letter that he received from a friend who was traveling in Central America was quoted by Anderson (2) to report the occurrence of smallpox in monkeys in their native habitat:

"In the year 1841 I was in the province of Veragua, in New Granada, to the north of the Isthmus of Panama, and left the town of St. Jago on the western coast for David in Chiriqui, a town in the interior, about sixty or seventy miles to the northeast (and leeward) of St. Jago. The smallpox was raging with great violence in St. Jago, but there was no appearance of it in David. A few days after my arrival there, taking my customary morning's ride in the forest, which teems with animal life, I was struck by observing one or two sick and apparently dying monkeys on the ground under the trees. The next morning I was struck by the same singular appearance (for it is very unusual to find a wild animal sick—they instinctively hide themselves) and, by thinking that I perceived several on the trees moping and moving about in a sickly manner, I consequently dismounted and carefully examined two, which were on the ground—one dead and the other apparently dying; and, after careful examination, no doubt remained in my mind that they were suffering and had died from smallpox. They presented every evidence of the disease, the pustules were perfectly formed, and in one instance (that of the dying one) the animal was nearly quite blind from the effects. A few days afterwards (I think about four or five days) the first case of smallpox appeared amongst the inhabitants of David, and in the course of a fortnight one-half of the population was stricken."

A point of interest in this letter, if one assumes that the sequence of events is valid, is the occurrence of smallpox in the native population after the disease was observed in monkeys. Admittedly, this is scant evidence that smallpox, as it exists in the simian host, is transmissible to man. Nevertheless, the danger of man acquiring smallpox from monkeys becomes credible in view of findings which indicated that monkeys infected with air-borne variola virus liberate the infectious agent from the respiratory passages during the incubation and disease periods (38). In addition, virus continued to be isolated from the upper respiratory tract for several days after the exanthem had disappeared.

Of further significance is the possibility that partially immune animals may harbor the virus in the respiratory tract without manifesting overt symptoms, or may exhibit only a modified form of the disease. Because smallpox, as it is manifested in the simian, is mild and of variable symptomatology, the signs of infection may not be readily distinguished. Indeed, confirmation of smallpox in animals known to have been exposed to variola virus often requires the use of virological and serological tests.

The possibility emerges, from an appraisal of present evidence, that monkeys entering a country from an endemic smallpox area, such as India, may serve as a source of infection for man. A modified or even an active form of smallpox infection in animals, being difficult to recognize, may create a hazard of infection for personnel who handle these animals in transit or on arrival in laboratory areas. Fortunately, through some physical or biological impediment of the sequence, there has been no recorded instance of human infection acquired in this manner. Nevertheless, cognizance of this situation by public health personnel may be judicious.

V. Comment

The simian host in experimental studies with the poxviruses, particularly smallpox virus, may provide a useful means whereby our understanding of the varied aspects of the disease process and its treatment, and evaluation of the immune state following vaccination may be increased. The rapid development in the past decade of tissue culture and fluorescent antibody methodology, if applied to the poxviruses in combination with the simian host, could conceivably answer the enigma of the different degrees of pathogenicity and virulence exhibited by the varied strains of variola and alastrim viruses. The marked similarity between smallpox infections in man and in monkeys (38) makes the latter host suitable for experimental studies that otherwise could not be performed.

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